Further *in vitro* studies of the effects of muscarinic agonists were made. Slices of chick cerebral hemispheres were pre-incubated for 30 min in Krebs-bicarbonate buffer. In the presence of the potent phosphodiesterase inhibitor papaverine (0.05 mM) the concentration of cyclic GMP in the slices (12–14 pmol/mg protein) was 2-fold higher than in its absence. Moreover exposure to oxotremorine (10 μM) produced a further increase of 100% in cyclic GMP only in the presence of papaverine.

From these results it is clear that the factors regulating cerebral cyclic GMP concentration differ from those controlling cyclic AMP. These initial studies show that in chick cerebral tissue, muscarinic agonists and prostaglandin $F_{2\alpha}$ stimulate cyclic GMP formation, but whether or not these effects result from the direct stimulation of cell surface receptors remains to be established.

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The effect of chronic lithium administration on stimulation-induced changes in forebrain 5-hydroxyindoles: modification by chlorimipramine

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Collard & Roberts (1974) showed that chlorimipramine reduced the production of 5-hydroxyindoleacetic acid (5-HIAA) by stimulation of the medial raphe nucleus, probably by inhibiting reuptake of neuronally released 5-hydroxytryptamine (5-HT). Using this method of examining 5-HT release this study reports the effect of lithium (Li^+).

Male Albino Wistar rats weighing 150–250 g were divided into two groups of 36 animals. One group received a daily injection of isotonic (0.15 M) LiCl (0.75 mEq/kg i.p.) for 10 days while the control group received saline. Twenty-four hours after the last dose of Li^+ or saline, half the animals in each group received chlorimipramine (5 mg/kg i.p.), while the others received saline. From each of the four

Table 1 The effect of chlorimipramine (5 mg/kg i.p.) on the stimulation-induced changes in forebrain 5-hydroxyindole concentrations in control animals and in animals which had received 10 day Li^+ treatment. Results are expressed as the mean ± s.e. mean of 9 pairs of animals and analysed by the paired t test. (+ and − indicate stimulation-induced increases and decreases, respectively in 5-hydroxyindole concentration).

Stimulation-induced change in 5-HT concentration (ng/g)

	Saline	*Chlorimipramine*	*Chlorimipramine minus saline*	*P*
Control	+44 ± 53	+116 ± 59	+72 ± 99	n.s.
Lithium	+71 ± 50	−42 ± 51	−113 ± 48	0.05

Stimulation-induced change in 5-HIAA concentration (ng/g)

	Saline	*Chlorimipramine*	*Chlorimipramine minus saline*	*P*
Control	+93 ± 12	−16 ± 15	−109 ± 20	0.001
Lithium	+98 ± 21	+78 ± 14	−20 ± 22	n.s.

subgroups, paired animals received raphe stimulation or sham treatment (Collard & Roberts, 1974).

Results are expressed as the difference between unstimulated and stimulated animals (Table 1). This difference does not appear to reflect drug induced changes in 5-hydroxyindole levels of unstimulated animals. Only the combination of Li^+ and chlorimipramine significantly altered changes in 5-HT levels, but Li^+ abolished the effect of chlorimipramine on 5-HIAA.

The reduction of 5-HIAA by chlorimipramine alone may indicate that the 5-HIAA produced by raphe stimulation arises predominantly from the metabolism of extraneuronally released 5-HT. Chlorimipramine by inhibiting uptake would deny released 5-HT access to intraneuronal monoamine oxidase and reduce the production of 5-HIAA.

Preliminary studies have indicated that Li^+ does not antagonize the inhibition of uptake by chlor imipramine. The lack of effect of chlorimipramine on the increase in 5-HIAA concentration in the Li^+ group suggests therefore that the 5-HIAA is produced primarily from 5-HT which remains intracellular. Since stored 5-HT is believed to be protected from deamination, the results may imply that Li^+ inhibits the extraneuronal release of 5-HT by promoting the release of 5-HT into the cytoplasm.

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The individual and combined effects of hypothermia and reserpine pretreatment on the rate and tension responses to isoprenaline and salbutamol in guinea-pig atria

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The dose-response curves for the positive inotropic and chronotropic effects on the heart of sympathomimetic amines have been shown to be displaced to the left by hypothermia, indicating supersensitivity (Trendelenburg, 1968; Reinhardt, Wagner & Schumann, 1972). Reserpine pretreatment has also been shown to produce supersensitivity of the rate (Taylor, Westfall & Fleming, 1974), and tension (McNeill & Shulze, 1972) responses, together with an increase in the maximum responses of the partial agonist salbutamol (Broadley & Lumley, 1975). We were therefore interested to determine the individual and combined effects of hypothermia and reserpine pretreatment upon the rate and tension dose response curves to isoprenaline and salbutamol, and whether the maximum response to the latter was also enhanced by hypothermia.

Separated left and right guinea-pig atria were suspended in Krebs bicarbonate solution (50 ml) initially at 38°C, and gassed with CO_2:O_2 (5%:95%). The left atrium was paced electrically at 2 Hz and isometric tension recorded on a Devices M19 polygraph for the inotropic responses. Chronotropic responses were obtained by means of a ratemeter triggered by the isometric tension signal of the spontaneous right atrium. An initial cumulative dose-response curve to isoprenaline was obtained at 38°; 30°; or 25°C. A third curve to salbutamol at the same temperature was compared with the second isoprenaline curve corrected for loss in sensitivity, from control experiments. Increase rate and increase tension responses were plotted as a percentage of the isoprenaline maximum response. Results are expressed as ng/50 ml to produce 50% of the maximal response.

The rate dose response curve to isoprenaline (60.2 ± 7.6) at 38°C lay to the left of the tension curve (354.4 ± 86.4). On cooling the preparation to 30°C both rate and tension curves were displaced to the left, tension to a greater extent, and the curves became virtually superimposable (Rate 23.57 ± 4.9; Tension 26.0 ± 2.5). Cooling to 25°C resulted in a further shift, and the tension curve now lay to the left of rate (Rate 8.75 ± 2.2; Tension 6.1 ± 1.7). The salbutamol curves were similarly displaced to the left by cooling, and their maxima were progressively raised from 61.2 ± 6.5 and 21.6 ± 11.3 at 38°C, to 75.2 ± 12.0 and 56.0 ± 15.6 at 30°C, and to 86.6 ± 7.1 and $66.3\% \pm 17.8$ at 25° for rate and tension respectively.

In atria from guinea-pigs pretreated with reserpine (5 mg/kg i.p. at 72 h and 3 mg/kg i.p. at 48 and 24 h before sacrifice) supersensitivity was demonstrated at 38°C by a shift of the dose response curves to isoprenaline to the left. Tension was potentiated more than rate as shown by their respective ED_{50} values of 46.2 ± 7.1 and 18.8 ± 3.3. At 30°C, reserpine-induced supersensitivity occurred in excess of that already